THERAPEUTIC POTENTIAL OF ORAL TOLERANCE

Lloyd Mayer and Ling Shao

The immune system has the daunting task of distinguishing between self and non-self. The mucosal immune system, present along the respiratory, gastrointestinal and genitourinary tracts, has the additional burden of coexisting with an abundance of dietary antigens and lumenal bacterial flora. A key feature of the mucosal immune system is its ability to remain tolerant to these antigens while retaining the capacity to repel pathogens effectively. Furthermore, tolerance generated at mucosal surfaces can translate to a more generalized systemic tolerance — a characteristic of great therapeutic potential, but with many unforeseen complexities that are explored in this review.

CONTACT-SENSITIZING AGENT
A substance that results in a local inflammatory response to that substance following repeated cutaneous or subcutaneous exposure.

ORAL TOLERANCE
Active non-response to an antigen administered through the oral route.

The Mount Sinai School of Medicine, Immunobiology Center, 1 Gustave L. Levy Place, New York 10029, USA. Correspondence to L.M. e-mail: Lloyd.Mayer@mssm.edu
doi:10.1038/nri1370

Antigens elicit qualitatively distinct immune responses based on their portal of entry (TABLE 1). Introduction of antigen systemically, whether by injection (subcutaneous or intramuscular) or injury, leads to local infiltration of inflammatory cells and specific immunoglobulin production[1]. By contrast, antigens introduced at mucosal surfaces (such as the gastrointestinal and genitourinary tracts) elicit active inhibition of the immune response to those antigens, systemically. In 1946, Merill Chase[2] showed that oral administration of a CONTACT-SENSITIZING AGENT (2,4-dinitrochlorobenzene) did not lead to sensitization, but rather prevented the animal from eliciting an immune response to subsequent intracutaneous injections and cutaneous challenges (FIG. 1). The specific induction of these regulated responses by administration of antigen through the gastrointestinal tract is known as ORAL TOLERANCE.

In the decades since the landmark experiment by Chase[2], researchers have gained important insights into the mechanisms of tolerance. We now know that tolerance is a normal feature of the immune system at mucosal surfaces. Tolerance can also be generated through nasal[3] and airway[4] administration of antigen. Two related forms of tolerance have also been described. Direct injection of antigen into the portal vein can lead to systemic non-responsiveness to that antigen (portal tolerance)[5], as can inoculation of the anterior chamber of the eye, in a phenomenon known as anterior-chamber-associated immune deviation (ACAID)[6].

However, oral tolerance remains the most rigorously investigated form of tolerance. Moreover, because oral administration of antigen can lead to systemic unresponsiveness, it is a potentially powerful tool for the therapy of autoimmunity and chronic inflammatory conditions, and an attractive alternative to immunosuppressive medical interventions that have undesirable side-effects (such as steroids). Several successful therapeutic trials in animal models have supported a limited number of human clinical trials. Unfortunately, in humans, heterogeneity in the design and results of these trials have made efficacy difficult to discern. In this review, we attempt to identify those factors that are essential for the use of oral tolerance as a therapeutic approach. First, a brief overview of the underlying mechanisms of oral tolerance is provided, followed by a selected review of animal models of disease, highlighting key issues that are important for the induction of oral tolerance. Finally, a comprehensive survey of human clinical trials using oral tolerance is discussed with an emphasis on future directions for research.

The path to oral tolerance

The mucosal immune system of the gastrointestinal tract has several special anatomical and physiological characteristics to carry out its unique functions. A comprehensive review of these characteristics with particular respect to tolerance has been published recently[7]; so, only a short summary of these is presented here.

Table 1 | **Route of antigen administration affects immunological outcome**

Route of antigen administration	Usual outcome	References
Subcutaneous	Immunization	1
Intramuscular	Immunization	1
Injury	Immunization	1
Intravenous	Tolerance	120
Mucosal (oral, nasal and respiratory)	Tolerance	3,4
Portal vein	Tolerance	5
Anterior chamber of the eye	Tolerance	6

Introduction of antigen through different routes lead to distinct outcomes. Immunization is characterized by local inflammation and specific antibody production. Tolerance is characterized by inhibition of systemic immunity to the specific antigen being administered.

COMMENSAL BACTERIA
Any one of the harmless or beneficial bacteria that colonize the small and large intestine.

Similar to the skin, the intestinal epithelium is a barrier to COMMENSAL BACTERIA and potential pathogens. Unlike the skin, however, the intestine must also be permeable to water and other nutrients. The unique nature of the mucosal immune response might be the consequence of a large antigenic burden of dietary antigens and commensal bacteria that are in constant contact with the intestinal epithelium. Damaging immune responses to these beneficial and/or harmless antigens must be prevented to maintain the integrity of the gut and allow for controlled nutrient absorption. Defects in this regulated state, such as immune responses to normal commensal flora, are thought to be an important contributing factor in the development of Crohn's disease — an inflammatory disorder of the gastrointestinal tract. This delicate balance in the gut between tolerance to innocuous antigens and immunity to pathogens is histologically recognized as controlled or physiological inflammation. As seen in FIG. 2, the external environment is separated from the lamina propria, which contains the largest collection of lymphoid tissue in the body, by a single layer of epithelium. The immune system at this interface is poised to react to insults, but remains tolerant to harmless or beneficial antigens. The decision to initiate immunity or tolerance integrates signals from several sources. For example, danger signals can be transmitted by receptors for pathogen-associated molecular patterns (PAMPs) known as pattern-recognition receptors (PRRs). These receptors, including the Toll-like receptors[8] and the NOD (nucleotide-binding oligomerization domain) family[9] of proteins, detect common motifs that are present on pathogens and can initiate an inflammatory cascade. Interestingly, these pathways seem to be dampened in the gut. In addition, the route by which antigens cross the epithelial-cell barrier is likely to dictate the type of immune response that is generated.

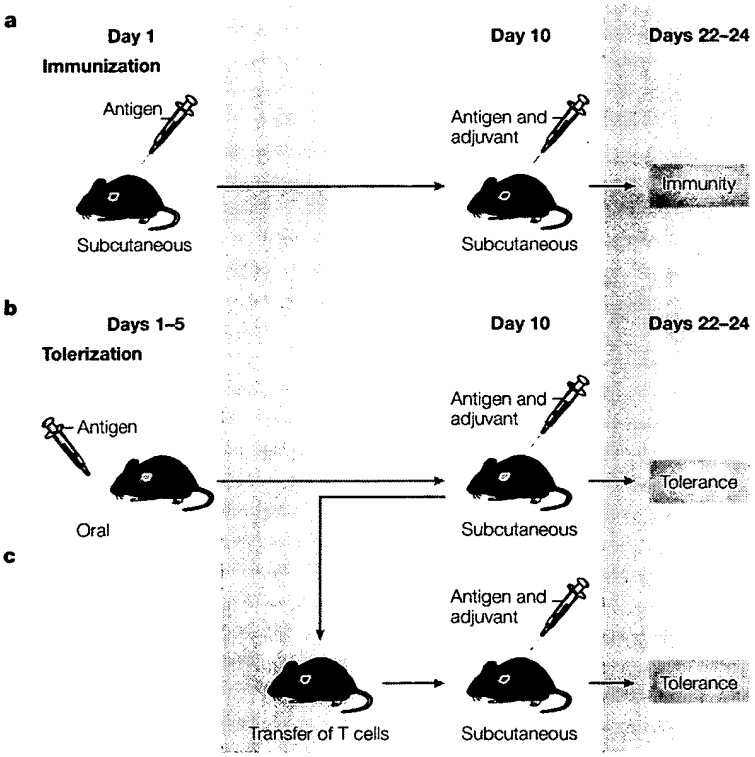

Figure 1 | **Induction of oral tolerance. a** | Mice that are immunized subcutaneously and then boosted subcutaneously with antigen plus adjuvant, such as Freund's complete adjuvant or alum, show a robust *in vitro* cell-mediated and antibody response to the immunizing antigen. **b** | Mice that are first orally fed antigen, then immunized subcutaneously with antigen plus an adjuvant have reduced immune responses to that antigen after *in vitro* restimulation. **c** | Finally, T cells from mice that are fed antigen (low dose) can be transferred to naive mice. Immunization of mice that received the tolerized T cells results in the same reduced response as seen in the mice that were fed antigen orally. This shows that oral feeding of antigen can induce an active (but inhibitory) immune response that is mediated by T cells.

In the normal non-diseased intestine, most antigens must pass through the large surface area of the intestinal epithelium. Estimates of the absorptive area of the human small bowel reach 200 m^2 (about the size of a tennis court)[10]. Intestinal epithelial cells might act as NON-PROFESSIONAL ANTIGEN-PRESENTING CELLS (APCs)[11,12] (FIG. 2) and have the capacity to modulate local immune responses through the activation of $CD8^+$ T cells[13]. Although in some circumstances $CD8^+$ T cells have been reported to mediate suppression after oral administration of antigens[14,15], many experiments have shown that $CD8^+$ T cells are dispensable for oral tolerance[16–18]. Antigens passing through intestinal epithelial cells might also be absorbed into capillaries that culminate in the portal vein, and early experiments in rats showed that surgical diversion of the portal vein (portal–caval shunt) abrogates tolerance[19]. Also, allogeneic tissue grafts are tolerated temporarily if donor splenocytes are injected through the portal vein simultaneously[20–22]. Mouse models indicate that the mechanism of portal tolerance might involve the presence of NK1.1$^+$ T CELLS[23]. So, one important pathway for tolerance might involve passing through intestinal epithelial cells, escaping capture by lamina-propria phagocytes and proceeding through blood capillaries to the liver.

Another important portal of entry for antigens are the organized lymphoid structures known as PEYER'S PATCHES (FIG. 2), which are distributed in the small bowel below specialized epithelial cells known as MICROFOLD CELLS (M cells). Although early studies supported a role for Peyer's patches in tolerance induction[24–26], more recent studies have cast doubt on this concept. M cells are specialized to take up particulate antigens, whereas peptide presentation of orally

administered soluble antigens occurs in the absence of Peyer's patches[27]. Mowat and colleagues[28,29] have shown that soluble antigens lead to systemic tolerance, whereas particulate antigens generally prime immune responses. Finally, ablation of Peyer's patches by genetic or pharmacological means does not affect tolerance[30].

Alternatively, it has recently been shown that intestinal dendritic cells can intercalate between epithelial cells and sample antigens directly from the lumen[31] (FIG. 2). Interestingly, expansion of dendritic-cell populations with FMS-LIKE TYROSINE KINASE 3 LIGAND (FLT3L) leads to an enhancement of oral tolerance[32]. Antigen-carrying dendritic cells traffic through the lymphatics (lacteals) to the mesenteric lymph nodes[33,34]. Genetic or pharmacological deletion of the mesenteric lymph nodes indicates that these structures are essential for oral tolerance, supporting a role for this pathway[35].

The pathways by which antigen either passes through the epithelium into blood capillaries, or is carried by phagocytic cells to the mesenteric lymph nodes through lymphatics (taken up through M cells or captured by dendritic cells) ultimately converge at the spleen. This

NON-PROFESSIONAL ANTIGEN-PRESENTING CELLS
Cells that can be induced to express antigen-presenting molecules or non-classical antigen-presenting molecules. These cells also often lack expression of co-stimulatory molecules such as CD80 and CD86.

NK1.1$^+$ T CELLS
Recently, these cells have been shown to be restricted by the non-classical MHC class Ib molecule CD1d. These cells respond to the antigens α-galactosylceramide and glycerol-phosphatidylinositol in mice and have important functions in immunity against infections and malignancies.

PEYER'S PATCHES
Organized lymphoid structures in the small intestine, underlying M cells. Peyer's patches consist of a T-cell zone surrounding a B-cell zone, similar to germinal centres in lymph nodes.

MICROFOLD CELLS
(M cells). Specialized epithelial cells that have a characteristic shape with a deep basolateral pocket and little cytoplasm. This makes them efficient at transporting insoluble materials across the epithelial-cell barrier, where these antigens immediately encounter macrophages and dendritic cells.

FMS-LIKE TYROSINE KINASE 3 LIGAND
(FLT3L). A cytokine that promotes the clonal expansion of dendritic cells *in vivo*.

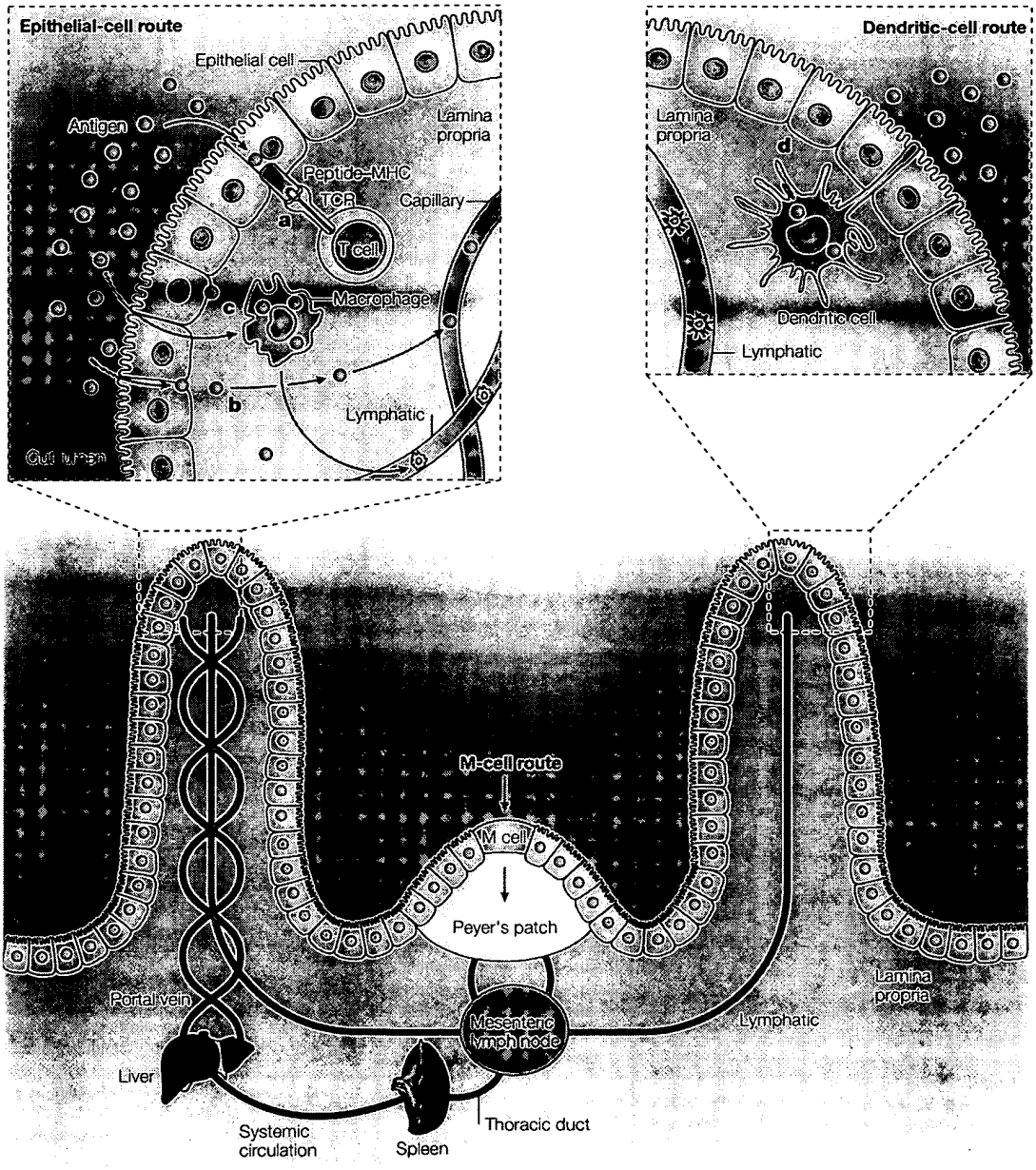

Figure 2 | **The path to oral tolerance.** Fed antigens can cross the intestinal epithelial-cell barrier in several ways. Antigens can be processed and presented on MHC molecules by intestinal epithelial cells (**a**) or cross through the epithelium, where they are absorbed into capillaries (**b**) that drain into the portal vein and the liver. Antigens can also be captured by macrophages (**c**) and carried to local draining lymph nodes in lymphatics. Dendritic cells have recently been shown to be capable of extending processes into the gut lumen (**d**), where they can capture antigen directly, which would then allow them to carry antigen to the local mesenteric lymph node in lymphatics. Each of these pathways ultimately converge at the spleen. It is possible that many redundant pathways exist to generate tolerance or that each individual pathway generates different forms of tolerance to specific antigens. TCR, T-cell receptor.

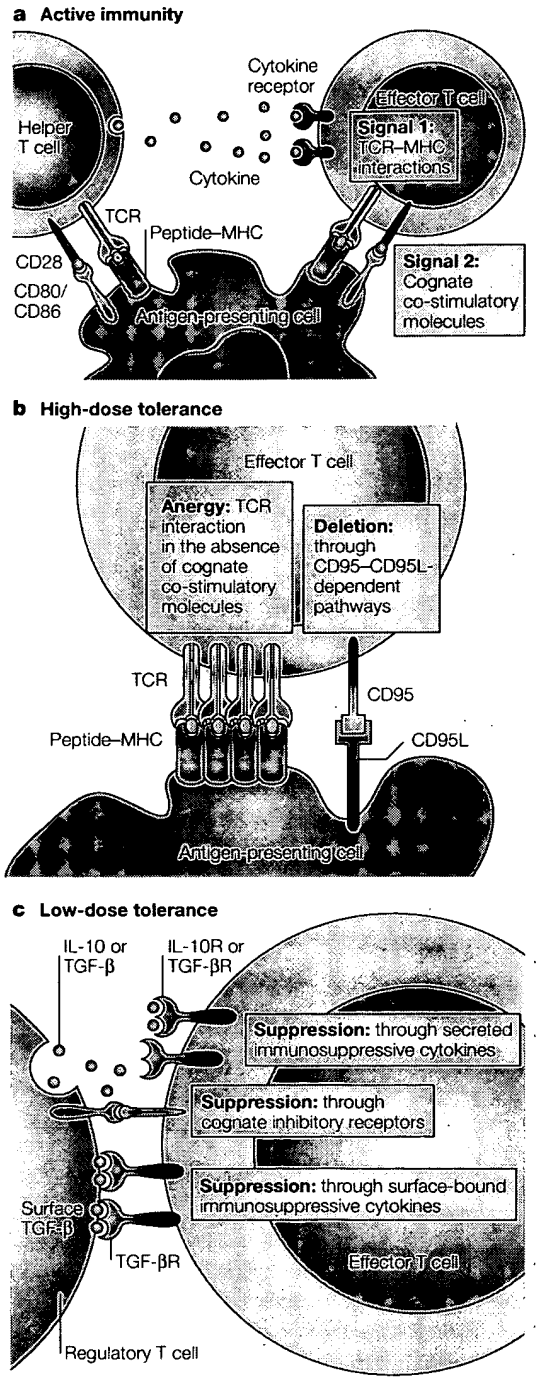

Figure 3 | **Potential mechanisms of oral tolerance.** **a** | The generation of an immune response requires ligation of the T-cell receptor (TCR) with peptide–MHC complexes. In the context of appropriate co-stimulatory molecules (CD80 and CD86) and cytokines, an active immune response is generated. **b** | However, with high doses of oral antigen, TCR crosslinking can occur in the absence of co-stimulation, or concurrently in the presence of inhibitory ligands (CD95 and CD95 ligand, CD95L), leading to immunosuppressive responses such as anergy or deletion. **c** | Low doses of oral antigen lead to the activation of regulatory T cells, which suppress immune responses by cognate interactions, and soluble (interleukin-10, IL-10, and transforming growth factor-β, TGF-β) or cell-surface-associated suppressive cytokines. R, receptor.

PERIPHERAL TOLERANCE
Potentially autoreactive T cells that have escaped negative selection in the thymus (central tolerance) can be deleted or anergized by one of several mechanisms. Deletion can be mediated by high-affinity T-cell receptor (TCR) crosslinking or by CD95–CD95L-mediated apoptosis. Anergy can occur when incomplete activation signals are sent through the TCR (low-affinity interactions) or when there is a lack of co-stimulation during activation.

ANERGY
A reversible immune hyporesponsiveness to antigen. Incomplete activation signals mediated by low-affinity T-cell receptor interactions or a lack of co-stimulation can lead to anergy. Anergy has been shown to be reversible by stimulation with antigen and interleukin-2.

would implicate the spleen as a potential site for tolerance induction. Some evidence indicates that splenectomy abrogates oral tolerance in a model of autoimmune uveitis[36]. Interestingly, absence of the spleen also eliminates ACAID[37,38]. So, different modes of tolerance might share common features.

High- and low-dose tolerance

In the 1960s, investigators studying the mechanisms of contact sensitization determined that systemic tolerance could be induced by administration of two widely separated doses of antigen[39]. In mice, we now know that oral tolerance occurs after either administration of a single high dose of antigen (>20 mg) or repeated exposure to lower doses (100 ng–1 mg)[40–42]. These two forms of tolerance, now termed high- and low-dose tolerance, are mediated by distinct mechanisms (FIG. 3).

Autoreactive cells that escape negative selection in the thymus (central tolerance) but then later encounter an abundance of self-antigen in the periphery may be either anergized or deleted in a process known as PERIPHERAL TOLERANCE. High doses of oral antigen can also induce lymphocyte ANERGY and/or deletion[43,44]. High-dose-induced deletion occurs by CD95 (FAS)-dependent caspase activation leading to apoptosis[45]. Interestingly, interleukin-12 (IL-12), a prototypical T helper 1 (T_H1)-cell-inducing cytokine, blocks the CD95 pathway and can block peripheral deletion in high-dose tolerance[46]. Anergy occurs through T-cell receptor (TCR) ligation with inadequate co-stimulation, either by cognate interactions between factors such as CD80 or CD86 on APCs with CD28 on T cells, or by soluble cytokines such as IL-2 (REF. 47).

Low-dose tolerance is now known to be mediated by active suppression of immune responses by T cells[48]. Specifically, the effector cells of low-dose tolerance have been shown by depletion, transfer and reconstitution studies to be mainly $CD4^+$ T cells[16,17,49]. However, these $CD4^+$ T cells defy the classical categorization of T_H1 and T_H2 cells and instead form a third broad grouping. Regulatory T cells that might have a role in oral tolerance can be divided into three subgroups: $CD4^+CD25^+$ regulatory T cells[48,50,51], T_H3 cells[52,53] and TR1 cells[54,55].

$CD4^+CD25^+$ regulatory T cells ($CD4^+$ T cells that co-express high levels of IL-2 receptor α-chain, CD25) mature in the thymus[56], are reactive to self-antigens in the periphery[57] and express the transcription factor forkhead box P3 (FOXP3)[58,59]. In humans, the importance of $CD4^+CD25^+$ regulatory T cells can be inferred from patients with a genetic defect in *FOXP3*. These patients develop a syndrome of immune dysregulation, polyendocrinopathy and enteropathy that is carried by the X chromosome (IPEX)[60]. In mice, the importance of $CD4^+CD25^+$ regulatory T cells in oral tolerance is indicated by experiments in transgenic mice that express a TCR specific for ovalbumin[48]. Ovalbumin-specific-TCR-transgenic mice that are fed ovalbumin have an increased number of $CD4^+CD25^+$ regulatory T cells. These cells were further shown to express high levels of cytotoxic T lymphocyte antigen 4 (CTLA4), as well as the immunosuppressive cytokines

Table 2 | **Oral tolerance in animal models of disease**

Animal model	Human disease	Induction	Effective oral antigens	Prophylactic or therapeutic	Dose	References
Experimental autoimmune encephalomyelitis	Multiple sclerosis	Commonly induced by injection of susceptible animals with myelin proteins plus pertussis toxin as an adjuvant to permeabilize the blood–brain barrier	Whole myelin, myelin basic protein, proteolipoprotein, myelin oligodendrocyte glycoprotein, glatiramer acetate (copolymer 1)	Prophylactic Prophylactic and therapeutic	High dose Low dose	42–44 63,94,95,125
Collagen-induced arthritis	Rheumatoid arthritis	Induced by injection of type II collagen in adjuvant	Collagen types II and IX, HSP65	Prophylactic	Low dose	74
Adjuvant-induced arthritis	Rheumatoid arthritis	Induced by injection of Freund's adjuvant, bacterial products or HSPs	HSP60, HSP65, type II collagen	Therapeutic	Low dose	114
Experimental autoimmune uveitis	Autoimmune uveitis	Induced by immunization with sequestered retinal antigens or IRBP	Retinal S-antigen, IRBP, HLA-B27 mimetope (HLA-B27PD)	Prophylactic	Low dose	78,79,97
Experimental autoimmune myasthenia gravis	Myasthenia gravis	Immunization with acetylcholine receptor	Acetylcholine receptor	Prophylactic	Low dose	77
Non-obese diabetic mice	Type 1 diabetes	Spontaneous destruction of pancreatic islet cells	Insulin	Prophylactic	Low dose	90,98,99,121
Rat insulin promoter LCMV diabetes model	Type 1 diabetes	Transgenic expression of LCMV proteins under the rat insulin promoter. Infection with LCMV initiates disease	Insulin	Prophylactic	Low dose	101
Middle cerebral artery occlusion	Stroke	Surgical occlusion of the middle cerebral artery	Myelin basic protein	Prophylactic	Low dose	64
LDL-receptor-deficient mice	Atherosclerosis	Mice lacking the LDL receptor are fed a high-fat diet	HSP65	Prophylactic	Low dose	65,66
Tissue transplant	Tissue transplant	Surgical transplantation of allogeneic tissue	Donor cells, donor MHC proteins	Prophylactic	Low dose	80,92

Several common models are used to study oral tolerance. Models are prophylactic if the regimen of oral feeding is begun prior to induction or onset of clinical disease, whereas they are therapeutic if oral tolerance is initiated after induction or onset of disease. Low doses correspond to <1 mg per day, whereas >1 mg per day is considered a high dose. This division is based on studies with myelin proteins in experimental autoimmune encephalomyelitis and might not accurately reflect doses with other antigens in other disease models. HSP, heat-shock protein; IRBP, interphotoreceptor retinoid-binding protein; LCMV, lymphocytic choriomeningitis virus; LDL, low-density lipoprotein.

IL-10 and transforming growth factor-β (TGF-β). Further work has shown that an important mechanism of action of $CD4^+CD25^+$ T cells might involve the expression of cell-surface-bound TGF-β, which can act as a cognate suppressive factor[61] (although other groups have not reproduced these findings).

TGF-β-secreting T_H3 cells have also been implicated in oral tolerance. These cells were found to be increased in patients with multiple sclerosis who are fed myelin compared with non-fed patients[52,53]. Finally, TR1 cells are activated by chronic alloantigen stimulation and require IL-10 for their growth as well as to suppress inflammation in mouse models of inflammatory bowel disease[54,55], but their contribution to oral tolerance is unclear. An intriguing feature shared by each of these subtypes of regulatory T cell is that, although they can be activated in an antigen-specific manner, based on their mechanism of action — suppressive cytokines and cell-surface ligands — they can suppress immune responses in the immediate surrounding area in an antigen non-specific manner. This phenomenon is known as BYSTANDER SUPPRESSION.

The ability to control this phenomenon is a potentially powerful therapeutic tool for controlling autoimmune/inflammatory conditions. Some examples of these are listed in TABLE 2.

BYSTANDER SUPPRESSION
Antigen non-specific inhibition of the immune response by virtue of temporal and physical proximity to regulatory cells.

Oral tolerance in animal models of human disease

A resurgence of interest in oral tolerance followed the finding that oral administration of autoantigens could prevent and/or ameliorate disease activity in animal models of rheumatoid arthritis[62] and multiple sclerosis[63]. Soon afterwards, investigators began to report similar results in other autoimmune diseases, as well as in models of allograft rejection. Oral tolerance has even shown beneficial effects in several non-autoimmune inflammatory applications such as atherosclerosis and stroke[64–66]. Several themes have emerged from these studies that might prove important for the application of oral tolerance in humans.

Dose. Independent of the model system, prevention of disease by low-dose oral tolerance occurs in a surprisingly narrow dose range. In experimental autoimmune encephalomyelitis (EAE) models, for example, prophylactic low doses of myelin basic protein (MBP) span a one log range (100–1000 μg)[40]. Higher doses of antigen can also protect a naive animal if administered before the onset or initiation of disease, probably through anergy or deletion of autoreactive cells[45,67]. Low doses are thought to be required to generate regulatory cells that are crucial for downmodulating ongoing inflammation[68].

Closely associated with the issue of dose is antigen digestion and absorption by the intestine. Barone and colleagues[69] showed that protecting the model antigen — in this case, ovalbumin — from digestion, by encapsulation in water-soluble acrylic microspheres, can interrupt established tolerance. Also, in a mouse model of food allergy, Untersmayr *et al.*[70] showed that mice had an increased propensity to food allergy when fed parvalbumin (derived from fish) together with an oral antacid. Tolerance might require gastric and gut lumenal pre-processing of antigen to create the appropriate tolerogenic epitopes, or alternatively, these studies might have involved the delivery of antigen doses that are unsuitable for tolerance induction in the intestine.

Antigen selection. Although the dose of antigen is crucial, the actual antigen used to induce tolerance can be variable in a particular disease. Several groups have now shown that feeding any one of the major myelin components — MBP, myelin oligodendrocyte glycoproteins (MOGs) or proteolipoprotein (PLP) — can protect susceptible animals from the development of EAE[71,72]. Models of type-II-collagen-induced arthritis can be ameliorated by feeding bacterial heat-shock protein 65 (HSP65)[73] or type IX collagen[74]. Models of adjuvant-induced arthritis can be prevented by the feeding of collagen[75].

Reinforcing the role of administration route in generating unique immune responses, even the immunodominant pathogenic peptide of MBP (NAc1–11) can act as a tolerogen when administered orally[76], as can the immunodominant epitopes of the acetylcholine receptor in experimental autoimmune myasthenia gravis[77] and retinal S-antigen in experimental autoimmune uveitis[78,79].

MBP has also been used to protect animals in a model of stroke. Inflammatory infiltration after transient local ischaemia might contribute to the pathogenesis of stroke. As regulatory T cells can act in an antigen non-specific manner, generating these cells against the abundant central nervous system antigen MBP might provide protection against inflammation in the brain by bystander suppression. Indeed, mice fed low doses of MBP have smaller infarcts after carotid occlusion than mice fed ovalbumin or immunized systemically with MBP[64].

In allogeneic transplants, donor MHC-derived peptides can prevent rejection of cardiac grafts[80]. Particularly in the case of allogeneic transplants, tolerogen flexibility is crucial. Although feeding allogeneic MHC antigens can prevent acute rejection, mismatched minor antigens can lead to chronic rejection[80,81]. To overcome this potential limitation, several groups have successfully induced graft tolerance by feeding a complex mixture of alloantigens such as donor splenocytes[82–86], donor bone-marrow cells[87], or donor epithelial or endothelial cells[81,88]. The myriad of antigens that can act as tolerogens indicates that developing generic therapies might be effective as an alternative to defining and using the specific autoantigen of the patient and allows for greater flexibility in preventing allograft rejection.

Altered peptide ligands and peptide analogues. MBP (NAc1–11)-specific TCR-transgenic mice are highly susceptible to the development of EAE after systemic immunization with MBP. Yet, the oral administration of NAc1–11 to these mice leads to tolerance rather than immunity[76]. Again, this experiment shows that the route of antigen administration clearly dictates the subsequent immune response. However, the mechanism by which the same cell can, in different microenvironments, mediate both disease and tolerance remains unclear. One possibility might be that the nature of the APC or the cytokine microenvironment is responsible for the distinct responses seen. Alternatively, the avidity of interaction between the TCR and peptide–MHC complexes can dictate the functional phenotype of the T cell.

In the periphery, high-avidity interactions between the TCR and peptide–MHC complexes can lead to the deletion of that T cell, whereas low-avidity contact is simply ignored. Optimal activation of a T cell resulting in clonal expansion and/or effector functions occurs in an intermediate range. Some evidence indicates that this paradigm also holds true for the induction of regulatory T cells[89]. Developing techniques to deliver a precise tolerogenic stimulus through the TCR might lead to more effective therapies.

One method to deliver the appropriate signal could be the use of closely related antigens or synthetically modified agents (altered peptide ligands and peptide analogues) that bind the TCR with slightly different avidity. Indeed, in two models of type 1 diabetes, non-obese diabetic (NOD) mice and RIP-LCMV mice (which express lymphocytic choriomeningitis virus proteins under control of the rat insulin promoter), the use of insulin β-chain derived from pigs, mice or humans protects these mice from disease with different efficiencies[90]. In a rat model of transplantation, Stepkowski *et al.*[91,92] created an altered peptide ligand by fusing donor and recipient MHC class I proteins. After transplant, T cells from rats fed the chimeric MHC molecules showed decreased phosphorylation of ζ-chain-associated protein of 70 kDa (Zap70; downstream of the TCR) and diminished IL-2-mediated activation of signal transducer and activator of transcription 5 (Stat5) compared with rats fed donor MHC molecules alone. Cells activated by chimeric MHC molecules consequently produced greater amounts of IL-4 and reduced levels of the pro-inflammatory cytokines interferon-γ (IFN-γ) and IL-2.

Synthetic peptides such as glatiramer acetate (copolymer 1) also have tolerogenic activity in mouse EAE models. Copolymer 1 seems to act as a structural analogue of the immunodominant MBP epitope NAc1–11 (REF. 93). Interestingly, the outcome of oral administration of copolymer 1 compared with the native MBP peptide is a marked increase in secretion of the immunosuppressive cytokines IL-10 and TGF-β, and a concurrent decrease in production of the inflammatory cytokines IL-2 and IFN-γ, and subsequently greater protection from EAE[94,95]. Overall, altered peptide ligands and peptide analogues show great promise as therapeutic alternatives to native antigens.

One caveat to translating these encouraging findings to human disease lies in the genetic heterogeneity that is present in humans but not in experimental systems. As Hafler's group[96] demonstrated, small variations in MHC alleles (polymorphisms) can lead to distinct functional outcomes with the same ligand. In this study, several T-cell clones were selected for strong proliferative and cytotoxic capacity against a peptide presented on HLA-A*0201. These clones when challenged with the peptide presented on closely related HLA-A2 molecules had diverse proliferative and cytotoxic responses. If similar genetic diversity can affect the generation of tolerogenic responses, designing altered peptide ligands or peptide analogues must also take into account HLA haplotypes.

Adjuvants. SYSTEMIC ADJUVANTS, such as alum or Freund's adjuvant, serve to enhance and propagate a peripheral immune response. In the setting of the tolerogenic mucosal immune system, an adjuvant might have the opposite effect to enhance inhibition of immune responses. One compound known to have this property is the B-subunit of cholera toxin.

The cholera holotoxin consists of two subunits, A and B. The A-subunit is responsible for the activation of adenyl cyclase, leading to ion and water secretion, and diarrhoea, whereas the cholera toxin B-subunit (CTB) targets the complex to a polysaccharide present on the intestinal epithelium (Gm1 ganglioside) and initiates internalization of the toxin. In addition, the B-subunit of cholera toxin alone, in contrast to the holotoxin (which is a potent mucosal adjuvant) or the A-subunit, promotes tolerance. Several groups have attempted to take advantage of this specific targeting to suppress inflammatory responses in animal models of disease.

The most striking example of the effectiveness of CTB was shown by Phipps *et al.*[97]; HSP60 can induce a form of experimental autoimmune uveitis when fed to genetically susceptible animals, but this is completely reversed by the conjugation of HSP60 to CTB. HSP60 conjugated to CTB activates IL-10- and TGF-β-producing cells in the mesenteric lymph nodes, which results in concomitant protection against disease.

Several groups have also shown that conjugating recombinant CTB to insulin increases the efficiency of tolerance induction. For example, studies in NOD mice have shown that oral administration of a CTB–insulin fusion protein induces IL-4- and TGF-β-secreting $CD4^+$ regulatory T cells and protects mice from spontaneous diabetes[98,99]. Also, pig, mouse and human insulin differ in their tolerogenic potency in the RIP-LCMV mouse model of type 1 diabetes. However, when conjugated to CTB, both pig and human insulin show increased, and now equal, effectiveness at inducing tolerance when administered within a narrow range of 1–5 μg twice a week[100,101].

Another approach in terms of mucosal adjuvants is to consider the mechanisms underlying low-dose tolerance. Immunosuppressive cytokines and T_H2/T_H3-type cytokines, such as IL-10, TGF-β and IL-4, have all been hypothesized to enhance tolerance, whereas inflammatory or T_H1-type cytokines, such as IL-12 or IFN-γ, have been thought to prevent or abrogate tolerance, as confirmed in mice with targeted gene defects[102,103]. Several recent reports show that administration of oral antigen leads to the activation of TGF-β-secreting regulatory cells[24,104–112], although genetically manipulated mice that lack TGF-β1 can be orally tolerized[113]. This indicates that both TGF-β-dependent and -independent mechanisms of tolerance exist. Further work must be carried out to clarify the contribution of each of these pathways to the phenomenon of oral tolerance.

Salbutamol (a β-adrenergic agonist) can inhibit IFN-γ production, while stimulating the production of IL-4 by T cells, whereas in macrophages, it stimulates IL-10 production and inhibits IL-12 production. These properties led Cobelens and colleagues[114] to study salbutamol in a rat model of adjuvant-induced arthritis. Co-administration of the antigen Hsp65 and salbutamol led to decreases in disease score as well as inflammatory cytokine production and T-cell proliferation compared with antigen alone. However, in contrast to all other known adjuvants, salbutamol does not augment the effect of feeding antigen before the induction of disease, but rather potentiates a therapeutic effect of oral Hsp65 after disease has been established.

Age, gender and genetic background. Because the age of onset of autoimmune and inflammatory diseases varies widely in humans, and allogeneic transplants can occur at almost any age, the role of age in oral tolerance induction is an important consideration.

Early studies showed that neonatal mice have defects in oral tolerance induction, possibly due to inherent defects in intestinal permeability (increased permeability)[115,116]. This is supported by studies in indomethacin-treated mice, which have microscopic mucosal lesions and subsequently a dose-dependent increase in intestinal permeability. Animals fed ovalbumin showed increased serum levels of the protein when treated with indomethacin and had a statistically significant reversal in systemic tolerance to ovalbumin[117]. However, more recent reports indicate that rapid antigen entry into the bloodstream after oral administration might actually enhance tolerance[118,119]. Tetramer technology was used to follow cytochrome-*c*-specific TCR-transgenic T cells after oral administration of antigen. After feeding of cytochrome *c*, T cells were rapidly redistributed (within 6 hours) from peripheral blood and mesenteric lymph nodes to the spleen and Peyer's patches and were resistant to *in vitro* antigen restimulation. Furthermore, orally administered antigen was found to be presented by splenocytes, possibly by naive B cells, within 6 hours. Interestingly, these data might be helpful in unifying oral tolerance with tolerance generated by rapid systemic entry of antigen by intravenous injection[120].

Maron *et al.*[121] showed that low doses of oral insulin protected neonatal NOD mice from developing spontaneous diabetes to a greater extent than in adult mice. However, this phenomenon was specific to the NOD

SYSTEMIC ADJUVANTS
Substances that help initiate a robust immune response. Typically, adjuvants contain a mixture of substances that mimic an active infection, such as bacterial cell-wall components to simulate danger signals and emulsifiers to allow for the slow release of antigen.

Table 3 | **Oral tolerance in human diseases**

Disease	Oral antigen	Dose	Prophylactic or therapeutic	Outcome	References
Food allergy	Allergen	Increasing dose over time	Therapeutic	About 80% of patients are successfully desensitized	130
Autoimmune uveitis	Sequestered retinal antigens, HLA-B27PD	4 mg capsules 3 times a week for 12 weeks	Therapeutic	Marginal clinical benefit. All patients relapsed after cessation of treatment	131,132
	Retinal S-antigen, soluble retinal antigens	30 mg S-antigen or 50 mg soluble retinal antigens or both. Decreasing dose, starting from 3 times a week for 8 weeks, ending with once a week	Therapeutic	No benefit, with possible exacerbation of disease in patients receiving a mixture of soluble retinal antigens	133
Rheumatoid arthritis	Collagen	0.1 mg bovine type II collagen daily for 1 month, followed by 0.5 mg daily for 6 months	Therapeutic	No benefit	135
		20, 100, 500 or 2,500 μg chicken type II collagen daily for 24 weeks	Therapeutic	Clinically significant response at 20 μg dose	136
		0.05, 0.5 or 5 mg bovine type II collagen daily for 6 months	Therapeutic	Response at 0.5 mg	137
		0.5 mg bovine type II collagen daily for 3 months	Therapeutic	Response at 0.5 mg	138
		0.1 mg chicken type II collagen daily for 1 month, followed by 0.5 mg for 2 months	Therapeutic	Improvement in most clinical measures, 4 out of 28 patients had complete remission	139
Type 1 diabetes	Insulin	7.5 mg insulin	Prophylactic	No benefit	
		2.5 mg or 7.5 mg insulin	Therapeutic	No benefit	140
Multiple sclerosis	Myelin	300 mg bovine myelin	Therapeutic	No clinically significant benefit	52,141

*For trial results see National Institutes of Health News website in Further Information. In contrast to experimental animal models, most human clinical trials have attempted to induce oral tolerance after the onset of disease (therapeutically). Treatments are prophylactic if the regimen of oral feeding is begun prior to the onset of clinical disease, whereas they are therapeutic if oral tolerance is initiated after the onset of disease. HLA-B27PD, HLA-B27 mimetope.

model, as oral feeding of PLP or MOG to neonates had no protective effect against the induction of EAE. In fact, in the EAE model, two groups have shown that early administration of MBP can exacerbate disease. Oral administration of MBP during the neonatal period to susceptible rats increased EAE clinical disease score[122]. This exacerbation slowly diminished if feeding was delayed. At the onset of adulthood (6 weeks of age), oral administration of MBP had a protective role against EAE induction. Melo *et al.*[123] described a similar enhancement of EAE in mice when guinea pig MBP was inoculated nasally during early life[123]. This immunostimulatory effect of nasal administration also gradually diminished in adult life and was indistinguishable from controls in 8–11-month-old mice. These studies highlight possible dangers in attempting oral tolerance induction in young children.

Many animal models, such as NOD mice, have a strong genetic predisposition to the development of disease. Analogous genetic variability might dictate tolerogenic immune responses as well. Russo *et al.*[124] compared the ability of two mouse strains that are susceptible to experimental asthma for their responsiveness to oral tolerance. BALB/c mice develop asthma in an IL-4-dependent manner, whereas BP2 mice develop airway hyperreactivity with high levels of IL-5 production and greater eosinophilia. Despite these differences, both strains of mice had suppressed allergic airway responses with decreased airway eosinophilia and immunoglobulin levels after oral feeding of allergen. These data indicate that, even in genetically complex diseases, treatment by oral tolerance might still be useful.

Most autoimmune diseases have a greater incidence in women, so gender differences might also have a role in oral tolerance. Bebo *et al.*[125] fed B10.PL mice with the MBP NAc1–11 peptide or a high-affinity analogue NAc1–11[Tyr4] and then attempted to induce EAE[125]. Female B10.PL mice are normally unresponsive to the induction of oral tolerance[126], but male mice showed marked protection when fed the high-affinity peptide analogue. Interestingly, this protection was lost if males were castrated prior to feeding. This indicates that sex hormones might also contribute to the phenomenon of oral tolerance.

Oral tolerance in human disease

Tolerance studies in normal healthy adults have been hindered by a lack of suitable neo-antigens. These antigens must be non-toxic, capable of being ingested and rarely encountered by the general population, such that immune responses can be compared between naive and orally tolerized individuals. Husby *et al.*[127] used keyhole limpet haemacyanin (KLH) to confirm the principle that oral tolerance to fed antigens could be established in humans. Eight adults were tolerized after ingestion of KLH at 50 mg per day for a total of 10 days. *In vitro* T-cell recall responses to KLH were inhibited, albeit with wide variability. Interestingly, serum immunoglobulin

titres of KLH-specific IgG and IgM, and secretory KLH-specific IgA titres seemed to be increased by the oral administration of KLH, demonstrating a dichotomy between the T- and B-cell responses to oral antigens. A second, more recent study[128] has confirmed that oral tolerance can be generated to orally administered antigen in normal humans. Together, these studies indicate that oral tolerance can be induced in humans, but indicate a greater benefit of oral tolerance in treating cell-mediated or T_H1-type inflammatory/autoimmune diseases, while potentially aggravating diseases, such as food allergy, systemic lupus erythematosus or glomerulonephritis, that are mediated by humoral or T_H2-type responses.

The obvious conclusion that can be drawn from the oral tolerance trials in normal human individuals and from the discussion of animal models is that the mechanisms behind oral tolerance are complex. Translation of oral tolerance from animal models to human disease must take into account not only issues relating to dose, antigen selection and age, but also genetic and environmental diversity. In addition, the effects of disease heterogeneity (genetic variability) and concomitant immunomodulatory medications must be considered. Indeed, the wide gap in the translation of studies in inbred mouse systems to humans has been confirmed by the limited number of human clinical trials carried out so far. In the following sections, we provide a comprehensive review of the use of oral tolerance in human diseases (TABLE 3). In all cases, except one (the diabetes prevention trial type 1, DPT-1), oral tolerance was used therapeutically. This is in contrast to most of the data from animal models discussed in the previous section, in which tolerance was induced prophylactically. Where possible, the lessons from both successes and failures are highlighted.

Food allergy. Several attempts have been made to lessen allergic responses by the feeding of allergen. The caveat here is that even minimal exposure to many food allergens (such as peanuts) can result in anaphylaxis. Classical immunotherapy protocols in food allergy use increasing doses of antigen that are administered subcutaneously, but the results of such approaches are variable[129]. In a recent clinical trial, allergen was fed to 59 patients with food allergy and their responses were compared with those of 16 controls who had not been treated[130]. The desensitization protocol used in this oral immunotherapy protocol involved an increasing dose of allergen, in some cases starting from nanogram quantities and increasing to gram quantities over the course of several months. Oral administration of a specific patient's allergen successfully desensitized ~80% of patients who could complete the study, whereas untreated patients showed no change in allergic status. No difference was found when patients were stratified by age. Finally, successfully treated patients could maintain the tolerogenic state by occasional oral exposure to the allergen.

This study indicates a possible role for oral tolerance in treating allergy, although the optimal dose to induce tolerance to individual allergens still needs to be determined. This could potentially decrease the length of time necessary for desensitization. Indeed, over the course of treatment in this study[130], 12 out of 59 patients were lost due to poor compliance.

Autoimmune uveitis. One small uncontrolled trial for the treatment of uveitis by administration of a MIMETOPE of sequestered retinal antigens (HLA-B27PD) showed marginal clinical improvement among nine patients given 4 mg of encapsulated peptide three times a week for 12 weeks[131,132]. However, all patients relapsed after the cessation of treatment. Together with the previous study, these data indicate that once tolerance is established in humans, it must be maintained by continued exposure to the antigen.

Two additional trials have also been carried out using oral tolerance in uveitis. The first trial tested 45 patients with purified retinal S-antigen, soluble retinal antigens or a combination of the two compared with placebo[133], whereas in the second trial, small doses of collagen (60 μg and 540 μg) were fed to 13 patients with uveitis associated with juvenile rheumatoid arthritis[134]. Neither of these trials showed a statistically significant benefit, nor were any toxic effects observed (although potential exacerbation of disease was found in one study[133] with patients fed the retinal mixture). Although the clinical benefit remains unclear, the second trial does suggest that oral tolerance can be safely used in children.

Rheumatoid arthritis. As suggested by mouse models, human studies in rheumatoid arthritis have highlighted the narrow dose range that can induce tolerance. One large multi-centre double-blind placebo-controlled trial showed no disease improvement in 190 patients that were fed 0.1 mg of bovine type II collagen daily for one month, followed by 0.5 mg per day for five months[135]. By contrast, Barnett *et al.*[136] showed a statistically and clinically significant effect at a dose of 20 μg but not 100 μg of chicken type II collagen. Additional smaller trials have shown some effectiveness at doses of 0.1 mg and 0.5 mg per day[137–139].

Differences in the results of these trials might be attributable to several factors: antigen source (bovine versus chicken type II collagen) and formulation, or concomitant use of medications. In the trial in which tolerance induction was unsuccessful[135], patients were allowed to remain on all medications, including steroids and non-steroidal anti-inflammatory drugs (NSAIDs), as well as disease-modifying antirheumatic drugs (DMARDs). However, in the trials that showed some success[136–138], patients were required to cease all DMARDs but, in some cases, were allowed to continue steroid or NSAID use. Little is known about the effects of immunomodulatory medications on oral tolerance and no rigorous studies have been reported in humans. One report in mice, as discussed previously, showed a decrease in oral tolerance after treatment

MIMETOPE
An epitope that structurally resembles another. Epitopes are three-dimensional structural motifs recognized by antibodies or by T-cell receptors (in the context of MHC). Similar or identical three-dimensional structures can be created by different peptides or synthetic compounds and can mimic the activity of the original antigen.

Box 1 | **Unanswered questions in oral tolerance**

Therapeutic versus prophylactic
Can oral tolerance be used to treat human diseases (therapeutically) or only used prophylactically?

Disease specificity
How will disease-specific factors affect oral tolerance?
How can tolerance be better targeted to the site of disease (for example, the joints in rheumatoid arthritis)?
Would certain diseases be better treated by inducing tolerance through other routes of administration (for example, the use of anterior-chamber-associated immune deviation in autoimmune uveitis)?

Background genetics
Will oral tolerance have to be tailored to specific HLA haplotypes?
Do all individuals have an equal potential to generate regulatory T cells?
Is there an inherent defect in tolerance in individuals with autoimmune or chronic inflammatory conditions because of other background genes (for example, co-stimulatory molecules)?

Optimal doses for each antigen
Is the optimal dose the same or different for each antigen (for example, myelin versus insulin versus synthetic peptides)?
Are certain antigens more tolerogenic?

Bystander suppression and other mechanisms of tolerance
Does bystander suppression exist in humans?
What are the antigen-presenting cells involved in tolerance induction? Dendritic cells, B cells, macrophages, epithelial cells or others?
What are the effector cells and mechanisms involved in oral tolerance? Regulatory-cell subsets; contact-dependent and -independent mechanisms of suppression; innate immune mediators?

Other factors
What are the effects of immunomodulatory factors or pharmaceuticals on oral tolerance: adjuvants (Flt3 ligand, salbutamol or cholera toxin, for example), steroids or non-steroidal anti-inflammatory drugs?

with the non-steroidal drug indomethacin, potentially due to increased intestinal permeability caused by the drug[117]. Until more information is available, interpretations of failed trials will be incomplete.

Type 1 diabetes. Although the efforts described so far have focused on the therapeutic induction of low-dose tolerance, the DPT-1 chose to attempt prophylactic tolerance in genetically predisposed patients at moderate risk of developing type 1 diabetes (see Further Information website for trial results). Unfortunately, oral administration of insulin at 7.5 mg per day failed to reduce the risk of developing disease, although there was no exacerbation of disease either.

In patients diagnosed with diabetes, oral insulin also failed to show any clinical benefit. 131 patients who met the World Health Organisation criteria for type 1 diabetes were fed 2.5 mg or 7.5 mg of insulin or a placebo. No differences were found between any of the groups in any of the diabetes parameters measured in this study[140].

Multiple sclerosis. Attempts to use oral tolerance for the treatment of multiple sclerosis held much promise based on the results of studies in EAE, but they have so far produced disappointing results in humans. An initial trial in 30 patients who were fed capsules containing 300 mg of bovine myelin showed no differences between myelin-fed and placebo-fed patients[141]. Another trial found increases in the number of TGF-β-secreting cells in peripheral blood of patients given oral myelin, but this objective measure did not correlate with a significant clinical benefit[52]. Subcutaneous injection of the peptide analogue, copolymer 1, has been shown, in several large clinical trials, to be effective in preventing relapses and reducing the severity of clinical disease, when compared with the placebo[142]. It remains to be seen whether oral copolymer 1 shows any efficacy in treating patients with multiple sclerosis.

Concluding remarks

Successful use of oral tolerance in treating human diseases will require a more detailed understanding of the underlying mechanisms behind the phenomenon. Although the field continues to progress rapidly, several key issues remain poorly understood and need to be addressed before this approach is ready for human use. Some of these are listed in BOX 1.

The key issue remains the translation of what is known in mouse models to human subjects. Tolerance is not synonymous in both species. Some studies in humans have indicated that the immune response can be deviated after the feeding of antigen, but this clearly needs to be evaluated more rigorously. Although studies in animal models can provide valuable clues, only direct studies in human subjects will provide appropriate answers.

In addition, some caution must be taken to prevent the aggravation or even initiation of disease. For example, in a model of type 1 diabetes, the oral administration of autoantigen induced disease[143]. Using transgenic mice that express ovalbumin under the rat insulin promoter, the investigators created a susceptible host by transferring T cells expressing a transgenic TCR specific for ovalbumin (OT-1 cells) to these mice. These mice did not spontaneously develop disease, but rather developed diabetes only after ingesting a 20 mg dose of ovalbumin. Experimentally, this model resembles the prophylactic high doses given to moderate-risk individuals in the DPT-1. However, oral administration of insulin in the DPT-1 did not lead to increased development of diabetes in these genetically susceptible individuals. Still, some evidence from human clinical trials supports caution. In the allergy trial carried out by Patriarcha *et al.*[130] discussed above, 9 out of 47 patients treated orally with allergen withdrew due to uncontrollable allergic complications.

Future work must clearly and carefully elucidate mechanisms of induction and maintenance of tolerance. Furthermore, defined animal models must be more meticulously translated to normal human subjects. Despite the disappointments in human clinical trials, oral administration of antigen to prevent autoimmune/inflammatory disease, as well as to delay tissue-graft rejection, remains a worthwhile and tantalizing goal.

1. Janeway, C. A., Jr, Travers, P., Walport, M. & Shlopov, B. V. *Immunobiology*. (Garland Publishing, New York, 2001).
2. Chase, M. W. Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. *Proc. Soc. Exp. Biol.* **61**, 257–259 (1946).
A landmark paper regarding the existence of oral tolerance and the fact that different routes of administration lead to distinct immune responses.
3. Boyaka, P. N. *et al.* Therapeutic manipulation of the immune system: enhancement of innate and adaptive mucosal immunity. *Curr. Pharm. Des.* **9**, 1965–1972 (2003).
4. Macaubas, C., DeKruyff, R. H. & Umetsu, D. T. Respiratory tolerance in the protection against asthma. *Curr. Drug Targets Inflamm. Allergy* **2**, 175–186 (2003).
5. Knolle, P. A. & Gerken, G. Local control of the immune response in the liver. *Immunol. Rev.* **174**, 21–34 (2000).
6. Stein-Streilein, J. & Streilein, J. W. Anterior chamber associated immune deviation (ACAID): regulation, biological relevance, and implications for therapy. *Int. Rev. Immunol.* **21**, 123–152 (2002).
7. Mowat, A. M. Anatomical basis of tolerance and immunity to intestinal antigens. *Nature Rev. Immunol.* **3**, 331–341 (2003).
8. Janssens, S. & Beyaert, R. Role of Toll-like receptors in pathogen recognition. *Clin. Microbiol. Rev.* **16**, 637–646 (2003).
9. Inohara, N. & Nunez, G. NODs: intracellular proteins involved in inflammation and apoptosis. *Nature Rev. Immunol.* **3**, 371–382 (2003).
10. *Report of the Task Group on Reference Man*. (Eds Snyder, W. S. *et al.*) (Pergamon, New York, 1975).
11. van de, W. Y. *et al.* Delineation of a CD1d-restricted antigen presentation pathway associated with human and mouse intestinal epithelial cells. *Gastroenterology* **124**, 1420–1431 (2003).
12. Allez, M., Brimnes, J., Dotan, I. & Mayer, L. Expansion of CD8$^+$ T cells with regulatory function after interaction with intestinal epithelial cells. *Gastroenterology* **123**, 1516–1526 (2002).
13. Grdic, D., Hornquist, E., Kjerrulf, M. & Lycke, N. Y. Lack of local suppression in orally tolerant CD8-deficient mice reveals a critical regulatory role of CD8$^+$ T cells in the normal gut mucosa. *J. Immunol.* **160**, 754–762 (1998).
14. Chen, Y., Inobe, J. & Weiner, H. L. Induction of oral tolerance to myelin basic protein in CD8-depleted mice: both CD4$^+$ and CD8$^+$ cells mediate active suppression. *J. Immunol.* **155**, 910–916 (1995).
15. Lider, O., Santos, L. M., Lee, C. S., Higgins, P. J. & Weiner, H. L. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. II. Suppression of disease and *in vitro* immune responses is mediated by antigen-specific CD8$^+$ T lymphocytes. *J. Immunol.* **142**, 748–752 (1989).
16. Barone, K. S., Jain, S. L. & Michael, J. G. Effect of *in vivo* depletion of CD4$^+$ and CD8$^+$ cells on the induction and maintenance of oral tolerance. *Cell. Immunol.* **163**, 19–29 (1995).
17. Garside, P., Steel, M., Liew, F. Y. & Mowat, A. M. CD4$^+$ but not CD8$^+$ T cells are required for the induction of oral tolerance. *Int. Immunol.* **7**, 501–504 (1995).
18. Vistica, B. P. *et al.* CD8 T cells are not essential for the induction of 'low-dose' oral tolerance. *Clin. Immunol. Immunopathol.* **78**, 196–202 (1996).
19. Callery, M. P., Kamei, T. & Flye, M. W. The effect of portacaval shunt on delayed-hypersensitivity responses following antigen feeding. *J. Surg. Res.* **46**, 391–394 (1989).
20. Fan, T. X. *et al.* Successful allogeneic bone marrow transplantation (BMT) by injection of bone marrow cells via portal vein: stromal cells as BMT-facilitating cells. *Stem Cells* **19**, 144–150 (2001).
21. Jin, T. *et al.* A novel strategy for organ allografts using sublethal (7 Gy) irradiation followed by injection of donor bone marrow cells via portal vein. *Transplantation* **71**, 1725–1731 (2001).
22. Wrenshall, L. E. *et al.* Modulation of immune responses after portal venous injection of antigen. *Transplantation* **71**, 841–850 (2001).
23. Trop, S. *et al.* Liver-associated lymphocytes expressing NK1. 1 are essential for oral immune tolerance induction in a murine model. *Hepatology* **29**, 746–755 (1999).
24. Santos, L. M., al Sabbagh, A., Londono, A. & Weiner, H. L. Oral tolerance to myelin basic protein induces regulatory TGF-β-secreting T cells in Peyer's patches of SJL mice. *Cell. Immunol.* **157**, 439–447 (1994).
The first demonstration that TGF-β has an important role in the induction of oral tolerance. Importantly, TGF-β is also the switch factor for the mucosal immunoglobulin soluble IgA.
25. Jeurissen, S. H., Sminia, T. & Kraal, G. Selective emigration of suppressor T cells from Peyer's patches. *Cell. Immunol.* **85**, 264–269 (1984).
26. Ngan, J. & Kind, L. S. Suppressor T cells for IgE and IgG in Peyer's patches of mice made tolerant by the oral administration of ovalbumin. *J. Immunol.* **120**, 861–865 (1978).
27. Kunkel, D., Kirchhoff, D., Nishikawa, S., Radbruch, A. & Scheffold, A. Visualization of peptide presentation following oral application of antigen in normal and Peyer's patches-deficient mice. *Eur. J. Immunol.* **33**, 1292–1301 (2003).
28. Mowat, A. M. The role of antigen recognition and suppressor cells in mice with oral tolerance to ovalbumin. *Immunology* **56**, 253–260 (1985).
29. Mowat, A. M., Thomas, M. J., Mackenzie, S. & Parrott, D. M. Divergent effects of bacterial lipopolysaccharide on immunity to orally administered protein and particulate antigens in mice. *Immunology* **58**, 677–683 (1986).
30. Spahn, T. W. *et al.* Induction of oral tolerance to cellular immune responses in the absence of Peyer's patches. *Eur. J. Immunol.* **31**, 1278–1287 (2001).
31. Rescigno, M. *et al.* Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nature Immunol.* **2**, 361–367 (2001).
32. Viney, J. L., Mowat, A. M., O'Malley, J. M., Williamson, E. & Fanger, N. A. Expanding dendritic cells *in vivo* enhances the induction of oral tolerance. *J. Immunol.* **160**, 5815–5825 (1998).
33. Scheinecker, C., McHugh, R., Shevach, E. M. & Germain, R. N. Constitutive presentation of a natural tissue autoantigen exclusively by dendritic cells in the draining lymph node. *J. Exp. Med.* **196**, 1079–1090 (2002).
34. Huang, F. P. *et al.* A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J. Exp. Med.* **191**, 435–444 (2000).
35. Spahn, T. W. *et al.* Mesenteric lymph nodes are critical for the induction of high-dose oral tolerance in the absence of Peyer's patches. *Eur. J. Immunol.* **32**, 1109–1113 (2002).
36. Suh, E. D. *et al.* Splenectomy abrogates the induction of oral tolerance in experimental autoimmune uveoretinitis. *Curr. Eye Res.* **12**, 833–839 (1993).
37. Takahashi, M. *et al.* Requirement for splenic CD4$^+$ T cells in the immune privilege of the anterior chamber of the eye. *Clin. Exp. Immunol.* **116**, 231–237 (1999).
38. Streilein, J. W. & Niederkorn, J. Y. Induction of anterior chamber-associated immune deviation requires an intact, functional spleen. *J. Exp. Med.* **153**, 1058–1067 (1981).
39. Mitchison, N. A. Induction of immunological paralysis in two zones of dosage. *Proc. R. Soc. Lond. B Biol. Sci.* **161**, 275–292 (1964).
40. Friedman, A. & Weiner, H. L. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Proc. Natl Acad. Sci. USA* **91**, 6688–6692 (1994).
The first study to raise the issue that dose of antigen can determine the type of tolerance generated (low doses result in suppression; high doses result in anergy/deletion).
41. Yoshida, T., Hachimura, S. & Kaminogawa, S. The oral administration of low-dose antigen induces activation followed by tolerization, while high-dose antigen induces tolerance without activation. *Clin. Immunol. Immunopathol.* **82**, 207–215 (1997).
42. Faria, A. M. *et al.* Oral tolerance induced by continuous feeding: enhanced upregulation of transforming growth factor-β/interleukin-10 and suppression of experimental autoimmune encephalomyelitis. *J. Autoimmun.* **20**, 135–145 (2003).
43. Bitar, D. M. & Whitacre, C. C. Suppression of experimental autoimmune encephalomyelitis by the oral administration of myelin basic protein. *Cell. Immunol.* **112**, 364–370 (1988).
44. Whitacre, C. C., Gienapp, I. E., Orosz, C. G. & Bitar, D. M. Oral tolerance in experimental autoimmune encephalomyelitis. III. Evidence for clonal anergy. *J. Immunol.* **147**, 2155–2163 (1991).
45. Chen, Y. *et al.* Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature* **376**, 177–180 (1995).
46. Marth, T., Zeitz, Z., Ludviksson, B., Strober, W. & Kelsall, B. Murine model of oral tolerance. Induction of Fas-mediated apoptosis by blockade of interleukin-12. *Ann. NY Acad. Sci.* **859**, 290–294 (1998).
47. Appleman, L. J. & Boussiotis, V. A. T cell anergy and co-stimulation. *Immunol. Rev.* **192**, 161–180 (2003).
48. Zhang, X., Izikson, L., Liu, L. & Weiner, H. L. Activation of CD25$^+$CD4$^+$ regulatory T cells by oral antigen administration. *J. Immunol.* **167**, 4245–4253 (2001).
49. Weiner, H. L. Induction and mechanism of action of transforming growth factor-β-secreting T_H3 regulatory cells. *Immunol. Rev.* **182**, 207–214 (2001).
50. Thorstenson, K. M. & Khoruts, A. Generation of anergic and potentially immunoregulatory CD25$^+$CD4$^+$ T cells *in vivo* after induction of peripheral tolerance with intravenous or oral antigen. *J. Immunol.* **167**, 188–195 (2001).
51. Dubois, B. *et al.* Innate CD4$^+$CD25$^+$ regulatory T cells are required for oral tolerance and inhibition of CD8$^+$ T cells mediating skin inflammation. *Blood* **102**, 3295–3301 (2003).
52. Fukaura, H. *et al.* Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor-β1-secreting T_H3 T cells by oral administration of myelin in multiple sclerosis patients. *J. Clin. Invest* **98**, 70–77 (1996).
53. Inobe, J. *et al.* IL-4 is a differentiation factor for transforming growth factor-β secreting T_H3 cells and oral administration of IL-4 enhances oral tolerance in experimental allergic encephalomyelitis. *Eur. J. Immunol.* **28**, 2780–2790 (1998).
54. Foussat, A. *et al.* A comparative study between T regulatory type 1 and CD4$^+$CD25$^+$ T cells in the control of inflammation. *J. Immunol.* **171**, 5018–5026 (2003).
55. Groux, H. *et al.* A CD4$^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* **389**, 737–742 (1997).
This study identifies a unique subset of regulatory T cells, TR1 cells, which are IL-10-secreting regulatory T cells.
56. Jordan, M. S. *et al.* Thymic selection of CD4$^+$CD25$^+$ regulatory T cells induced by an agonist self-peptide. *Nature Immunol.* **2**, 301–306 (2001).
57. Cozzo, C., Larkin, J., III & Caton, A. J. Self-peptides drive the peripheral expansion of CD4$^+$CD25$^+$ regulatory T cells. *J. Immunol.* **171**, 5678–5682 (2003).
58. Khattri, R., Cox, T., Yasayko, S. A. & Ramsdell, F. An essential role for Scurfin in CD4$^+$CD25$^+$ T regulatory cells. *Nature Immunol.* **4**, 337–342 (2003).
59. Fontenot, J. D., Gavin, M. A. & Rudensky, A. Y. Foxp3 programs the development and function of CD4$^+$CD25$^+$ regulatory T cells. *Nature Immunol.* **4**, 330–336 (2003).
60. Bennett, C. L. *et al.* The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nature Genet.* **27**, 20–21 (2001).
References 59 and 60 identify a novel transcription factor (FOXP3) that is associated with a major subset of regulatory T cells (CD4$^+$CD25$^+$).
61. Nakamura, K. *et al.* TGF-β1 plays an important role in the mechanism of CD4$^+$CD25$^+$ regulatory T cell activity in both humans and mice. *J. Immunol.* **172**, 834–842 (2004).
62. Nagler-Anderson, C., Bober, L. A., Robinson, M. E., Siskind, G. W. & Thorbecke, G. J. Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen. *Proc. Natl Acad. Sci. USA* **83**, 7443–7446 (1986).
The first demonstration in an animal model that feeding of the immunogen before systemic exposure can abrogate disease. This is the foundation on which the human studies were developed.
63. Higgins, P. J. & Weiner, H. L. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein and its fragments. *J. Immunol.* **140**, 440–445 (1988).
64. Becker, K. J. *et al.* Immunologic tolerance to myelin basic protein decreases stroke size after transient focal cerebral ischemia. *Proc. Natl Acad. Sci. USA* **94**, 10873–10878 (1997).
65. Harats, D., Yacov, N., Gilburd, B., Shoenfeld, Y. & George, J. Oral tolerance with heat shock protein 65 attenuates *Mycobacterium tuberculosis*-induced and high-fat-diet-driven atherosclerotic lesions. *J. Am. Coll. Cardiol.* **40**, 1333–1338 (2002).
66. Maron, R. *et al.* Mucosal administration of heat shock protein-65 decreases atherosclerosis and inflammation in aortic arch of low-density lipoprotein receptor-deficient mice. *Circulation* **106**, 1708–1715 (2002).
67. Samoilova, E. B. *et al.* CTLA-4 is required for the induction of high dose oral tolerance. *Int. Immunol.* **10**, 491–498 (1998).
68. Chen, Y. *et al.* Oral tolerance in myelin basic protein T-cell receptor transgenic mice: suppression of autoimmune encephalomyelitis and dose-dependent induction of regulatory cells. *Proc. Natl Acad. Sci. USA* **93**, 388–391 (1996).
69. Barone, K. S., Reilly, M. R., Flanagan, M. P. & Michael, J. G. Abrogation of oral tolerance by feeding encapsulated antigen. *Cell. Immunol.* **199**, 65–72 (2000).
70. Untersmayr, E. *et al.* Antacid medication inhibits digestion of dietary proteins and causes food allergy: a fish allergy model in BALB/c mice. *J. Allergy Clin. Immunol.* **112**, 616–623 (2003).

71. al Sabbagh, A., Miller, A., Santos, L. M. & Weiner, H. L. Antigen-driven tissue-specific suppression following oral tolerance: orally administered myelin basic protein suppresses proteolipid protein-induced experimental autoimmune encephalomyelitis in the SJL mouse. *Eur. J. Immunol.* **24**, 2104–2109 (1994).
72. Karpus, W. J., Kennedy, K. J., Smith, W. S. & Miller, S. D. Inhibition of relapsing experimental autoimmune encephalomyelitis in SJL mice by feeding the immunodominant PLP139–151 peptide. *J. Neurosci. Res.* **45**, 410–423 (1996).
73. Jorgensen, C., Gedon, E., Jaquet, C. & Sany, J. Gastric administration of recombinant 65 kDa heat shock protein delays the severity of type II collagen induced arthritis in mice. *J. Rheumatol.* **25**, 763–767 (1998).
74. Myers, L. K. *et al.* Immunogenicity of recombinant type IX collagen in murine collagen-induced arthritis. *Arthritis Rheum.* **46**, 1086–1093 (2002).
75. Zhang, Z. Y., Lee, C. S., Lider, O. & Weiner, H. L. Suppression of adjuvant arthritis in Lewis rats by oral administration of type II collagen. *J. Immunol.* **145**, 2489–2493 (1990).
76. Song, F. *et al.* Differences between two strains of myelin basic protein (MBP) TCR transgenic mice: implications for tolerance induction. *J. Autoimmun.* **18**, 27–37 (2002).
77. Baggi, F. *et al.* Oral administration of an immunodominant T-cell epitope downregulates T_H1/T_H2 cytokines and prevents experimental myasthenia gravis. *J. Clin. Invest* **104**, 1287–1295 (1999).
78. Vrabec, T. R., Gregerson, D. S., Dua, H. S. & Donoso, L. A. Inhibition of experimental autoimmune uveoretinitis by oral administration of S-antigen and synthetic peptides. *Autoimmunity* **12**, 175–184 (1992).
79. Nussenblatt, R. B. *et al.* Inhibition of S-antigen induced experimental autoimmune uveoretinitis by oral induction of tolerance with S-antigen. *J. Immunol.* **144**, 1689–1695 (1990).
80. Zavazava, N. *et al.* Oral feeding of an immunodominant MHC donor-derived synthetic class I peptide prolongs graft survival of heterotopic cardiac allografts in a high-responder rat strain combination. *J. Leukoc. Biol.* **67**, 793–800 (2000).
81. He, Y. G., Mellon, J. & Niederkorn, J. Y. The effect of oral immunization on corneal allograft survival. *Transplantation* **61**, 920–926 (1996).
82. Holan, V. *et al.* Induction of specific transplantation immunity by oral immunization with allogeneic cells. *Immunology* **101**, 404–411 (2000).
83. Ilan, Y. *et al.* Induction of oral tolerance in splenocyte recipients toward pretransplant antigens ameliorates chronic graft versus host disease in a murine model. *Blood* **95**, 3613–3619 (2000).
84. Ishido, N., Matsuoka, J., Matsuno, T., Nakagawa, K. & Tanaka, N. Induction of donor-specific hyporesponsiveness and prolongation of cardiac allograft survival by jejunal administration of donor splenocytes. *Transplantation* **68**, 1377–1382 (1999).
85. Nagler, A. *et al.* Oral tolerization ameliorates liver disorders associated with chronic graft versus host disease in mice. *Hepatology* **31**, 641–648 (2000).
86. Niederkorn, J. Y. & Mayhew, E. Phenotypic analysis of oral tolerance to alloantigens: evidence that the indirect pathway of antigen presentation is involved. *Transplantation* **73**, 1493–1500 (2002).
87. Gorczynski, R. M., Chen, Z., Zeng, H. & Fu, X. M. A role for persisting antigen, antigen presentation, and ICAM-1 in increased renal graft survival after oral or portal vein donor-specific immunization. *Transplantation* **66**, 339–349 (1998).
88. Ma, D., Mellon, J. & Niederkorn, J. Y. Oral immunisation as a strategy for enhancing corneal allograft survival. *Br. J. Ophthalmol.* **81**, 778–784 (1997).
89. Suto, A. *et al.* $CD4^+CD25^+$ T-cell development is regulated by at least 2 distinct mechanisms. *Blood* **99**, 555–560 (2002).
90. Homann, D., Dyrberg, T., Petersen, J., Oldstone, M. B. & Von Herrath, M. G. Insulin in oral immune 'tolerance': a one-amino acid change in the B chain makes the difference. *J. Immunol.* **163**, 1833–1838 (1999).
91. Stepkowski, S. M. *et al.* Allochimeric class I MHC protein-induced tolerance by partial TCR engagement requires activation of both CTL4- and common γ-chain-dependent cytokine signals. *Transplantation* **73**, 1227–1235 (2002).
92. Stepkowski, S. M., Yu, J., Wang, M. & Kahan, B. D. Induction of tolerance by oral administration of a tolerogenic allochimeric donor/recipient class I MHC protein. *Transplant. Proc.* **31**, 1557 (1999).
93. Aharoni, R., Teitelbaum, D., Arnon, R. & Sela, M. Copolymer 1 acts against the immunodominant epitope 82-100 of myelin basic protein by T cell receptor antagonism in addition to major histocompatibility complex blocking. *Proc. Natl Acad. Sci. USA* **96**, 634–639 (1999).
94. Maron, R., Slavin, A. J., Hoffmann, E., Komagata, Y. & Weiner, H. L. Oral tolerance to copolymer 1 in myelin basic protein (MBP) TCR transgenic mice: crossreactivity with MBP-specific TCR and differential induction of anti-inflammatory cytokines. *Int. Immunol.* **14**, 131–138 (2002).
95. Teitelbaum, D., Arnon, R. & Sela, M. Immunomodulation of experimental autoimmune encephalomyelitis by oral administration of copolymer 1. *Proc. Natl Acad. Sci. USA* **96**, 3842–3847 (1999).
96. Lim, D. G., Slavik, J. M., Bourcier, K., Smith, K. J. & Hafler, D. A. Allelic variation of MHC structure alters peptide ligands to induce atypical partial agonistic $CD8^+$ T cell function. *J. Exp. Med.* **198**, 99–109 (2003).
97. Phipps, P. A. *et al.* Prevention of mucosally induced uveitis with a HSP60-derived peptide linked to cholera toxin B subunit. *Eur. J. Immunol.* **33**, 224–232 (2003).
98. Bergerot, I. *et al.* A cholera toxoid–insulin conjugate as an oral vaccine against spontaneous autoimmune diabetes. *Proc. Natl Acad. Sci. USA* **94**, 4610–4614 (1997).
99. Ploix, C. *et al.* Oral administration of cholera toxin B–insulin conjugates protects NOD mice from autoimmune diabetes by inducing $CD4^+$ regulatory T-cells. *Diabetes* **48**, 2150–2156 (1999).
100. Bregenholt, S. *et al.* The cholera toxin B subunit is a mucosal adjuvant for oral tolerance induction in type 1 diabetes. *Scand. J. Immunol.* **57**, 432–438 (2003).
101. Petersen, J. S. *et al.* Coupling of oral human or porcine insulin to the B subunit of cholera toxin (CTB) overcomes critical antigenic differences for prevention of type I diabetes. *Clin. Exp. Immunol.* **134**, 38–45 (2003).
102. Rizzo, L. V. *et al.* IL-4 and IL-10 are both required for the induction of oral tolerance. *J. Immunol.* **162**, 2613–2622 (1999).
103. Mowat, A. M., Steel, M., Leishman, A. J. & Garside, P. Normal induction of oral tolerance in the absence of a functional IL-12-dependent IFN-γ signaling pathway. *J. Immunol.* **163**, 4728–4736 (1999).
104. Zemann, B. *et al.* Oral administration of specific antigens to allergy-prone infant dogs induces IL-10 and TGF-β expression and prevents allergy in adult life. *J. Allergy Clin. Immunol.* **111**, 1069–1075 (2003).
105. Sato, M. N. *et al.* Oral tolerance induction in *dermatophagoides pteronyssinus*-sensitized mice induces inhibition of IgE response and upregulation of TGF-β secretion. *J. Interferon Cytokine Res.* **21**, 827–833 (2001).
106. Lundin, B. S. *et al.* Active suppression in orally tolerized rats coincides with *in situ* transforming growth factor-β (TGF-β) expression in the draining lymph nodes. *Clin. Exp. Immunol.* **116**, 181–187 (1999).
107. Hafler, D. A. *et al.* Oral administration of myelin induces antigen-specific TGF-β1 secreting T cells in patients with multiple sclerosis. *Ann. NY Acad. Sci.* **835**, 120–131 (1997).
108. Haneda, K. *et al.* TGF-β induced by oral tolerance ameliorates experimental tracheal eosinophilia. *J. Immunol.* **159**, 4484–4490 (1997).
109. Strober, W. *et al.* Reciprocal IFN-γ and TGF-β responses regulate the occurrence of mucosal inflammation. *Immunol. Today* **18**, 61–64 (1997).
110. Ma, C. G. *et al.* Mucosal tolerance to experimental autoimmune myasthenia gravis is associated with downregulation of AChR-specific IFN-γ-expressing T_H1-like cells and upregulation of TGF-β mRNA in mononuclear cells. *Ann. NY Acad. Sci.* **778**, 273–287 (1996).
111. Neurath, M. F. *et al.* Experimental granulomatous colitis in mice is abrogated by induction of TGF-β-mediated oral tolerance. *J. Exp. Med.* **183**, 2605–2616 (1996).
112. Miller, A., al Sabbagh, A., Santos, L. M., Das, M. P. & Weiner, H. L. Epitopes of myelin basic protein that trigger TGF-β release after oral tolerization are distinct from encephalitogenic epitopes and mediate epitope-driven bystander suppression. *J. Immunol.* **151**, 7307–7315 (1993).
A study on bystander suppression in an oral tolerance model. This paper laid the foundation for the concept that the actual definition of the inciting agent/autoantigen in a patient was not important, as regulatory T cells activated in the area of pathology could suppress inflammation/autoreactivity.
113. Barone, K. S., Tolarova, D. D., Ormsby, I., Doetschman, T. & Michael, J. G. Induction of oral tolerance in TGF-β1 null mice. *J. Immunol.* **161**, 154–160 (1998).
114. Cobelens, P. M. *et al.* The β2-adrenergic agonist salbutamol potentiates oral induction of tolerance, suppressing adjuvant arthritis and antigen-specific immunity. *J. Immunol.* **169**, 5028–5035 (2002).
115. Strobel, S. & Ferguson, A. Immune responses to fed protein antigens in mice. III. Systemic tolerance or priming is related to age at which antigen is first encountered. *Pediatr. Res.* **18**, 588–594 (1984).
116. Strobel, S. & Ferguson, A. Modulation of intestinal and systemic immune responses to a fed protein antigen, in mice. *Gut* **27**, 829–837 (1986).
117. Louis, E. *et al.* Decrease in systemic tolerance to fed ovalbumin in indomethacin-treated mice. *Int. Arch. Allergy Immunol.* **109**, 21–26 (1996).
118. Gutgemann, I., Darling, J. M., Greenberg, H. B., Davis, M. M. & Chien, Y. H. A blood-borne antigen induces rapid T–B cell contact: a potential mechanism for tolerance induction. *Immunology* **107**, 420–425 (2002).
119. Gutgemann, I., Fahrer, A. M., Altman, J. D., Davis, M. M. & Chien, Y. H. Induction of rapid T cell activation and tolerance by systemic presentation of an orally administered antigen. *Immunity* **8**, 667–673 (1998).
120. Liblau, R., Tisch, R., Bercovici, N. & McDevitt, H. O. Systemic antigen in the treatment of T-cell-mediated autoimmune diseases. *Immunol. Today* **18**, 599–604 (1997).
121. Maron, R., Guerau-de-Arellano, M., Zhang, X. & Weiner, H. L. Oral administration of insulin to neonates suppresses spontaneous and cyclophosphamide induced diabetes in the NOD mouse. *J. Autoimmun.* **16**, 21–28 (2001).
122. Miller, A., Lider, O., Abramsky, O. & Weiner, H. L. Orally administered myelin basic protein in neonates primes for immune responses and enhances experimental autoimmune encephalomyelitis in adult animals. *Eur. J. Immunol.* **24**, 1026–1032 (1994).
123. Melo, M. E., Stevens, D. B., Sercarz, E. E. & Gabaglia, C. R. Nasal instillation of gpMBP can exacerbate murine EAE: effect of mucosal priming is an age-dependent phenomenon. *J. Autoimmun.* **22**, 13–20 (2004).
124. Russo, M. *et al.* Suppression of asthma-like responses in different mouse strains by oral tolerance. *Am. J. Respir. Cell Mol. Biol.* **24**, 518–526 (2001).
125. Bebo, B. F., Jr. *et al.* Gender differences in protection from EAE induced by oral tolerance with a peptide analogue of MBP-Ac1–11. *J. Neurosci. Res.* **55**, 432–440 (1999).
126. Metzler, B. & Wraith, D. C. Mucosal tolerance in a murine model of experimental autoimmune encephalomyelitis. *Ann. NY Acad. Sci.* **778**, 228–242 (1996).
127. Husby, S., Mestecky, J., Moldoveanu, Z., Holland, S. & Elson, C. O. Oral tolerance in humans. T cell but not B cell tolerance after antigen feeding. *J. Immunol.* **152**, 4663–4670 (1994).
The first study to show that oral tolerance exists in humans. Tolerance was shown in T cells but not B cells, a finding that distinguishes tolerance in humans from that seen in mice.
128. Kraus, T. A., Toy, L., Chan, L., Childs, J. & Mayer, L. Failure to induce oral tolerance to a soluble protein in patients with inflammatory bowel disease. *Gastroenterology* (in the press).
129. Eigenmann, P. A. Future therapeutic options in food allergy. *Allergy* **58**, 1217–1223 (2003).
130. Patriarca, G. *et al.* Oral desensitizing treatment in food allergy: clinical and immunological results. *Aliment. Pharmacol. Ther.* **17**, 459–465 (2003).
131. Thurau, S. R., Diedrichs-Mohring, M., Fricke, H., Burchardi, C. & Wildner, G. Oral tolerance with an HLA-peptide mimicking retinal autoantigen as a treatment of autoimmune uveitis. *Immunol. Lett.* **68**, 205–212 (1999).
132. Thurau, S. R., Diedrichs-Mohring, M., Fricke, H., Arbogast, S. & Wildner, G. Molecular mimicry as a therapeutic approach for an autoimmune disease: oral treatment of uveitis-patients with an MHC-peptide crossreactive with autoantigen – first results. *Immunol. Lett.* **57**, 193–201 (1997).
133. Nussenblatt, R. B. *et al.* Treatment of uveitis by oral administration of retinal antigens: results of a phase I/II randomized masked trial. *Am. J. Ophthalmol.* **123**, 583–592 (1997).
134. Thompson, D. J., Barron, K. S., Whitcup, S. M. & Robinson, M. R. The safety and efficacy of chicken type II collagen on uveitis associated with juvenile rheumatoid arthritis. *Ocul. Immunol. Inflamm.* **10**, 83–91 (2002).
135. McKown, K. M. *et al.* Induction of immune tolerance to human type I collagen in patients with systemic sclerosis by oral administration of bovine type I collagen. *Arthritis Rheum.* **43**, 1054–1061 (2000).
136. Barnett, M. L. *et al.* Treatment of rheumatoid arthritis with oral type II collagen. Results of a multicenter, double-blind, placebo-controlled trial. *Arthritis Rheum.* **41**, 290–297 (1998).

137. Choy, E. H. *et al.* Control of rheumatoid arthritis by oral tolerance. *Arthritis Rheum.* **44**, 1993–1997 (2001).
138. Ausar, S. F. *et al.* Treatment of rheumatoid arthritis by oral administration of bovine tracheal type II collagen. *Rheumatol. Int.* **20**, 138–144 (2001).
139. Trentham, D. E. *et al.* Effects of oral administration of type II collagen on rheumatoid arthritis. *Science* **261**, 1727–1730 (1993).
140. Chaillous, L. *et al.* Oral insulin administration and residual β-cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial. Diabete Insuline Orale group. *Lancet* **356**, 545–549 (2000).
141. Weiner, H. L. *et al.* Double-blind pilot trial of oral tolerization with myelin antigens in multiple sclerosis. *Science* **259**, 1321–1324 (1993).
142. Wolinsky, J. S., Narayana, P. A. & Johnson, K. P. United States open-label glatiramer acetate extension trial for relapsing multiple sclerosis: MRI and clinical correlates. Multiple Sclerosis Study Group and the MRI Analysis Center. *Mult. Scler.* **7**, 33–41 (2001).
143. Blanas, E., Carbone, F. R., Allison, J., Miller, J. F. & Heath, W. R. Induction of autoimmune diabetes by oral administration of autoantigen. *Science* **274**, 1707–1709 (1996).

Acknowledgements
This work is supported by the National Institutes of Health.

Competing interests statement
The authors declare that they have no competing financial interests.

Online links

DATABASES
The following terms in this article are linked online to:
EntrezGene: http://www.ncbi.nlm.nih.gov/Entrez/
CD28 | CD80 | CD86 | CD95 | CTLA4 | FOXP3 | IL-2 | IL-10 | IL-12 | TGF-β | Zap70

FURTHER INFORMATION
National Institutes of Health News:
http://www.nih.gov/news/pr/jun2003/niddk-15.htm
Lloyd Mayer's homepage:
http://adsr13.mssm.edu/domains/dept/facultyInfo.epl?objname=immunobiology&user=mayerl01&sid=12809_830
Access to this interactive links box is free online.